IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	CLEARY, et al.
Serial No.:	10/529,622
Filed:	March 30, 2005
For:	Highly Purified Amphotericin B
Group Art Unit:	1623
Examiner:	PRESELEV, Elli
Attorney's Docket No.	11636N/020724
Customer No.	32885

DECLARATION UNDER 37 C.F.R. § 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

I, Robert E. Kramer, Ph.D., declare:

1. I have significant experience in the field of pharmacology. My current position is Professor, Department of Pharmacology and Toxicology, University of Mississippi Medical Center. I consider myself to be one of ordinary skill in the art.

2. I have read and understood the Office Action mailed April 23, 2007, in connection with the above-identified application. Furthermore, I have read and understand the patents cited therein.

3. The Office Action mailed April 23, 2007 states that "applicant has not provided any evidence in verified form showing that the references' [Michel et al., US '789 and Tang '375] purification process would not result in a product having the claimed purity levels."

4. US '789, relied upon by the outstanding Office Action in connection with purification issues, discloses a four solvent system for purifying amphotericin B: methanol, dimethylformamide, methylene chloride, and water. In US '789, purity is measured as "residue on ignition." Even an implied purity of 99.9% based on residue after ignition (≤0.1%) is not a valid or appropriate measurement in terms of the claimed compositions (comprising at least 94% amphotericin B and 4% or less of impurity products). Ignition *per se* is not a quantitative means of estimating purity. If the 'ignition test' was done properly, any organic compound consisting of carbon, hydrogen and nitrogen – e.g., amphotericin B and other polyene contaminants – would be expected by one in the field to ignite completely. Thus, under proper conditions, the residue remaining after ignition is an index of inorganic or metal content and not of purity of the organic material. One of ordinary skill in the art would understand that the residue on

ignition, or results therefrom, should not be used to argue that the product of that method was essentially a single molecular species. In other words, this test could not confirm the presence of, or lack of, the harmful polyene/endotoxin contaminants addressed by the present invention.

5. There is no indication that a product made from the methods disclosed in US '789 would be any different from those currently commercially available in terms of non-amphotericin B polyene and/or endotoxin content.

6. US '375, relied upon by the outstanding Office Action in connection with purification issues, discloses a method of purifying amphotericin B with an ion exchange column, removing gram positive and gram negative bacteria. From a review of this reference, one of ordinary skill in the art would understand that the disclosed ion exchange column process would not result in the features claimed. In other words, this purification step should not result in a composition with the claimed purity characteristics.

7. There is no indication that a product made from the methods disclosed in US '375 that a product would be any different from those currently commercially available in terms of non-amphotericin B polyene and/or endotoxin content.

8. The present inventors discovered that the purification process described in the present specification and the products made therefrom are substantially more pure that what is currently available. The present inventors discovered the need for such a purification method and products made therefrom. Accordingly, the compositions of the present invention allows for reduced adverse reactions.

9. The Office Action further requests a showing of "unexpected results relating to obtaining the subject compound having the claimed purity levels." In connection with obtaining the subject compositions having the claimed purity levels, the claimed compounds are superior. This result is unexpected.

10. The claimed compositions of this invention can be made by separating amphotericin BHP from contaminants in a commercially available amphotericin B formulation. That separation is achieved by the use of High Performance Liquid Chromatography or HPLC as presented in Example 6, page 15. In comparison to liquid:liquid extraction, precipitation and recrystallization, chromatography (or HPLC as described in example 6), adds another dimension for the separation of physically and chemically similar compounds such as amphotericin B and other polyene contaminants that are co-extracted from bacterial cultures: distance. This was not known in the art with respect to reducing adverse amphotericin B reactions.

11. Chromatography, in general, is based on the differential interaction of compounds dissolved in a liquid phase with a solid phase on the basis of their relative solubility or other physiochemical characteristics. Separation of the dissolved compounds occurs as the liquid phase is allowed to flow in a designated direction, while the solid phase is kept stationary. In example 6, the liquid phase is 70% methanol:30% 5 mM sodium citrate (pH 7), whereas the stationary phase is the 4.6 x 150 mm AquaC18 column. The 150 mm is the column length or distance over which separation of amphotericin B and other contaminants is achieved. HPLC specially enhances the separation though the use of a solid phase that consists of small particles of uniform size (≤10 microns) with pores of uniform diameter (≤10 Angstroms); both characteristics increase the surface area over which the dissolved substances and solid phase interact. The effectiveness of the separation is determined by monitoring (for example, by ultraviolet absorption) the liquid phase as it elutes from the column.

12. The ability of HPLC as described in example 6 to separate amphotericin B (i.e., amphotericin BHP) from other polyene compounds present in a commercially available amphotericin B formulation is illustrated in Figure 1 (HPLC FRACTIONS) of this application, which depicts the absorbance of column eluate at 305 nm and 405 nm. In addition, Figure 4 (below) illustrates the apparent purity of amphotericin BHP that can be

achieved by the method described in example 6 using USP-grade amphotericin B as the parent material. In this illustration, the amphotericin BHP product has an apparent purity of 98%, whereas the apparent purity of the USP-grade amphotericin B is 90%. The result in purity is superior and unexpected.

13. In comparison, two methods utilizing liquid:liquid extraction and recrystallization, again starting with USP-grade amphotericin B, did not result in comparable increases in apparent purity of the products as determined by analytical HPLC and absorbance at 405 nm.

Compound	Apparent Purity (%)
parent USP-grade amphotericin	93
product of recrystallization method 1	95
product of recrystallization method 2	89

14. The data presented in the table above argue strongly that the separation of amphotericin B from other chemically similar organic species would not be markedly improved through the use of comparable, if not identical methods. The data presented in the above table also illustrate an important limitation of previous 'state of the art' methods based on

liquid:liquid extraction and recrystallization. That limitation is the inability to separate chemically and structurally similar compounds such as amphotericin B and related polyenes beyond a given finite level. Embodiments of the present invention (i.e., HPLC as described in example 6) provides a clear advantage in that regard, offering a means to substantially improve apparent purity of amphotericin B. This method results in amphotericin B products not previously available, including those that comprise at least 94% amphotericin B and less than 4% or less impurities, and including those where the impurities are non-amphotericin B polyene compounds and/or endotoxin compounds.

15. The relationships between purity, efficacy and toxicity comprise well established principles of pharmacology and therapeutics, and the fact that the incidence of untoward reactions is inversely related to drug purity is an established pharmacological tenet. Thus, the superior and unexpected improvement in the apparent purity of amphotericin B that is of achieved with the present invention is magnified further if one accepts the basic premise that currently available USP-grade amphotericin B – the parent compound from which all currently marketed therapeutic formulations of amphotericin B are derived – is the product of the present 'state of the art'. From that perspective, the products of the present invention are superior to the forms of amphotericin B currently available.

16. The undersigned declares further that all statements made herein of his knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Signed this 29 day of October 2007.

Robert E. Kramer

Figure 4. High Performance Liquid Chromatographic Comparison of USP-Grade Amphotericin B (USP AmB) and Amphotericin B High Purity (AmBHP).